U.S. DEPARTMENT OF COMMERCE
National Technical Information Service
PB-278 592

Alterations in Bacterial Defense Mechanisms of the Lung Induced by Inhalation of Cadmium

Health Effects Research Lab, Research Triangle Park, N C

1977

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

MS-76-068

1. REPORT NO. EPA-600/J-77-089	2. JOURNAL ARTICLE
3. RE	PB 278 592
4. TITLE AND SUBTITLE: Alterations in Bacterial Defense Mechanisms of the Lung Induced by Inhalation of Cadmium	5. REPORT DATE
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): D.E. Gardner, F.J. Miller, J.W. Illing and J.M. Kirtz	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Clinical Studies Division, Health Effects Research Laboratory, Office of Research and Development, Research Triangle Park, N.C. 27711	10. PROGRAM ELEMENT NO. 1AA601
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS: Health Effects Research Laboratory RTP, NC, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA 600/11

15. SUPPLEMENTARY NOTES

Published in: Bulletin Europeen de Physiopathologie Respiratoire 13:157-174, 1977

16. ABSTRACT

Exposure to an aerosol of $CdCl_2$ has a marked proclivity to reduce the ability of the lung to defend itself against microbial insults. A significant enhancement of mortality was observed in mice exposed to $CdCl_2$ concentrations ranging from 80 to 1600 $\mu g/m^3$ prior to being challenged with viable streptococci. The increase in percent mortality above control varied from 15% at the lowest $CdCl_2$ concentration to approximately 70% at the highest concentration. In order to determine the various mechanistic factors which may explain the observed effect of reduced host resistance to infection, a variety of pulmonary defense systems was studied. There was a significant decrease in the total number of alveolar macrophages recoverable from rat lungs immediately after completion of the exposure regimen. The number of macrophages returned to normal (about 5.5 million) within 24 hours after cessation of the exposure. However, total polymorphonuclear leucocytes increased 1.5 million immediately after completion of the exposure, and 13 million within 24 hours after cessation of the exposure. Lymphocyte numbers were not affected by these exposure levels. Data concerning clearance of streptococci from the lung following $CdCl_2$ exposure closely correlated with the observed mortality pattern.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
cadmium infectious diseases respiratory diseases bacteria toxicity	defense mechanisms infectivity model	06 F

18. DISTRIBUTION STATEMENT: RELEASE TO PUBLIC	19. SECURITY CLASS (This Report): UNCLASSIFIED 20. SECURITY CLASS (This page): UNCLASSIFIED	21. NO. OF PAGES: 20 22. PRICE: PCA02 | MF A01

EPA Form 2220-1 (9-73)

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

IIIS-76-068

EPA-600/J-77-089
JOURNAL ARTICLE

Extrait du Bulletin européen de Physiopathologie Respiratoire
Volume 13 - 1977

ALTERATIONS IN BACTERIAL DEFENSE MECHANISMS OF THE LUNG INDUCED BY INHALATION OF CADMIUM

MODIFICATIONS DES MÉCANISMES DE DÉFENSE ANTIBACTÉRIENNE DU POUMON ENTRAINÉES PAR UNE INHALATION DE CADMIUM

D.E. Gardner, F.J. Miller, J.W. Illing, J.M. Kirtz *

Bull. europ. Physiopath. resp., 1977, *13*, 157-174.

ABSTRACT

Exposure to an aerosol of $CdCl_2$ has a marked proclivity to reduce the ability of the lung to defend itself against microbial insults. A significant enhancement of mortality was observed in mice exposed to $CdCl_2$ concentrations ranging from 80 to 1600 $\mu g/m^3$ prior to being challenged with viable streptococci. The increase in percent mortality above control varied from 15 % at the lowest $CdCl_2$ concentration to approximately 70 % at the highest concentration. In order to determine the various mechanistic factors which may explain the observed effect of reduced host resistance to infection, a variety of pulmonary defense systems was studied. There was a significant decrease in the total number of alveolar macrophages recoverable from rat lungs immediately after completion of the exposure regimen. The number of macrophages returned to normal (about 5.5 million) within 24 hours after cessation of the exposure. However, total polymorphonuclear leucocytes increased 1.5 million immediately after completion of the exposure, and 13 million within 24 hours after cessation of the exposure. Lymphocyte numbers were not affected by these exposure levels. Data concerning clearance of streptococci from the lung following $CdCl_2$ exposure closely correlated with the observed mortality pattern.

Alveolar macrophage ; cadmium ; pulmonary host defense ; respiratory disease.

INTRODUCTION

Understanding the role of trace metals in the environment and their interaction with biological systems is becoming increasingly important to the overall understanding of their potential hazard to health. Metals are some of the most insidious pollutants because of their non-biodegradable nature and due to the

* Biomedical Research Branch, Clinical Studies Division, Health Effects Research Laboratory, Research Triangle Park, North Carolina 27711, USA.

Reproduced from best available copy.

fact that some are chemically highly active. They are capable of undergoing chemical reactions themselves, of serving as catalysts [4], or of acting as vehicles for the transport of gaseous pollutants [2, 3].

Recent investigations have demonstrated that certain toxic metallic elements, including the one examined in this study, are closely associated with submicrometer sized particles [8, 23, 25]. Once these small particles are inhaled and deposited upon respiratory tissue, their impact at the cellular level becomes a major concern to the environmental toxicologist. In the alveolar region, the prime responsibility for clearing the body of inhaled substance is that of alveolar macrophages (AM) [17, 20, 21]. Any impairment in the functioning of these specialized cells may render the host more susceptible to inhaled infectious agents.

The study which will be reported here was designed for two reasons: 1) to determine if inhalation of one of these metals, $CdCl_2$, would potentiate laboratory induced respiratory infections and 2) to determine the possible disorders in the functioning in some respiratory defense systems which could account for this toxic manifestation.

METHOD

Exposure procedure

Animal inhalation exposures were performed using rats and mice. All animals received a single $CdCl_2$ exposure of two hours duration. Following the exposure, the animals were used in a variety of biological experiments in order to examine toxicological effects of this pollutant. These experiments included studies of effects on AM, deposition and clearance of viable and non-viable particles, susceptibility to pulmonary infection, and relative mean survival time.

The exposure chamber is a rectangular Plexiglas box having a height and width of one foot and a length of approximately eight feet. There are four ports on each of two sides to which Plexiglas modules containing animals can be attached. The modules for rats and mice consist of banks of cylindrical tubes. Each tube projects into the chamber, allowing the head of the animal to be exposed, and has numerous holes through which the test substance can circulate. The other end of the tube is plugged with a rubber stopper through which a Plexiglas plunger protrudes to keep the animal's head firmly in place at the chamber end of the tube. Each mouse and rat module can hold six and three animals, respectively. Clean filtered air is allowed to pass through the chamber for several minutes in order to permit the animals to become accustomed to their containment.

A chamber air flow of slightly more than one chamber volume/minute (approximately 9 ft^3/min) is maintained by a small fan whose speed is controlled with a voltage rheostat. This blower draws air from the chamber through an absolute filter to remove the $CdCl_2$ aerosol prior to exhausting the air into the atmosphere. The aerosol generation system supplies about 2.5 ft^3/min. of chamber air, while the remainder is supplied from an absolute filtered dilution source. Chamber pressure is maintained at approximately 0.35 inches of water below that of the room. Total air flow into the chamber is monitored with an orifice meter located between the exposure chamber and a 0.3 ft^3 baffled mixing chamber. Just inside the chamber entrance, a small circular baffle plate is mounted to spatially distribute the aerosol after it enters the chamber.

The aerosol is generated with a modified Environmental Research Corporation Fluid Atomization Aerosol Generator. The nebulizer flask was modified in the following manner to provide for circulation of the solution. A small pump pulls liquid from a side arm on the nebulizer flask, which maintains a constant level in the flask. The pump transfers the solution to a reservoir, from which it returns to the nebulizer flask. The purpose of this system is to reduce the effect of concentration of the nebulizer solution due to evaporation of water by effectively increasing the liquid capacity of the nebulizer flask.

Reproduced from best available copy.

Air from a Gast oil-less air compressor is filtered with a Wilkerson air line filter (Model 1137), regulated to 35 psi, and then passed through a silica gel bed. After passing through an absolute filter, the air is split into two streams, one of which is passed through a flowmeter and the nebulizer, and the other through a valve and flowmeter. The two streams are then recombined and the diluted aerosol is passed through a Krypton 85 ionizer to reduce the charge on the aerosol to equilibrium. Except for the compressor, all the above-mentioned components of the aerosol generating system are housed in a glove box that is maintained at a pressure approximately 0.3 of an inch of water below that of the room. From the outlet of the glove box, the aerosol stream enters the small mixing chamber to be further diluted before entering the exposure chamber.

To prepare the aerosol generation system for each exposure, water which has accumulated is removed from the air filter, and the silica gel is removed from the dryer and regenerated in an oven. Immediately prior to the start of an exposure, $CdCl_2$ is weighed and dissolved in deionized water in a 500 ml volumetric flask. Following the exposure, the nebulizer solution is removed and the system is rinsed with deionized water. The animal modules are cleaned between exposures, except in the few cases when two exposures are run on the same day.

Aerosol monitoring

The aerosol in the exposure chamber was monitored for mass concentration, as well as for size. Four to seven minute samples were pulled through 0.22 µm pore diameter filters (Millipore Corp.) at a rate of 2.65 l/min. A filter holder (Millipore No. XX50-047-00) with an attached 3.0 l/min orifice and a vacuum pump (Fisher No. 1-093-5) were used. The vacuum was sufficient to operate the orifice at its critical flowrate, which was determined with a wet test meter (Precision Scientific No. 63115). After collection of the sample, each filter was placed in a 125 ml Ehrlenmeyer flask, and 19.5 ml of deionized water and 0.5 ml of 6N HNO_3 were added. The flasks were placed on a mechanical shaker and agitated for two hours. Then several milliliters of each solution were poured into individual polyethylene vials and frozen until analysed.

Size analysis of the aerosol was conducted using a light scattering device (Royco Instruments Model 225 Aerosol Particle Monitor with Model 507 Module) which determines frequency counts for the following size ranges : 1) 0.5 - 0.6, 2) 0.6 - 1.4, 3) 1.4 - 3.0, 4) 3.0 - 5.0, and 5) 5.0 - 10.0 µm diameter. This analysis indicated that 94-99 % of the particles were smaller than 1.4 µm diameter and at least 99 % were smaller than 3.0 µm. The temperature and humidity of the air in the chamber were monitored using a Rustrak Model 225 Temperature and Humidity Recorder. The average chamber temperature was 25 °C with a standard error of 0.4 °C, while the relative humidity was 33.1 % ± 0.8 %.

Tissue analysis for inhaled Cd

Immediately, and at various times following a 2 h exposure, animals were randomly selected from each exposure group and sacrificed to determine Cd content of the lung. The lungs were excised, minced with stainless steel scissors, placed in a tared glass boat, and immediately weighed to determine the wet weight. The lungs were dried in an oven at 100 °C and 24 in. Hg vacuum for 48 h and then reweighed to determine the dry weight. Glass boats containing the tissues were placed in a low temperature asher (LFE Corp. Model LTA-505) for 48 h under the conditions of 400 watts forward radio-frequency (RF) power and 200 ml/min oxygen flow. The dried residue was then dissolved with 6N HNO_3 and the sample stored at 0 °C in polyethylene vials until analysed. Two ml of nitric acid were used for mouse lungs and 5 ml for rat lungs.

The analysis of tissue and filter samples was performed using an atomic absorption spectrophotometer with a heated graphite atomizer (Perkin Elmer Models 306 and 2100, respectively) under the following conditions : a wavelength of 2280 Å, a slit size of 7 Å, drying for 60 seconds at 100 °C, ashing for 50 seconds at 800 °C, atomizing for 9 seconds at 2100 °C, background correction. In order to prevent Cd loss during the ashing cycle, 10 µl of 20 % $(NH_4)_2$ HPO_4 were added to 10 µl of sample to form a Cd complex which would not vaporize at the ashing temperature. The method of « standard additions » was used

for calibrating the spectrophotometer. A multi-point calibration curve was generated each day samples were analysed. Standard solutions with concentrations between 5 and 100 μg/ml were made from a 1 mg/ml Cd stock solution (Fisher Certified Atomic Absorption Reagent). Since there often was curvature at the high end of the calibration curve, a least squares regression analysis for both a straight line and a quadratic equation was performed for each day's calibration points. Of the two resulting equations, the one which best fit the calibration points in the region of the experimental data was used. Both the signed magnitude of the residuals and their sequence were used in making this determination.

Bacterial exposure

Following the 2 h metal exposure, the animals were removed, combined with control animals, placed in a stainless steel chamber, and exposed either immediately or 24 h later to an aerosol of viable *Streptococcus pyogenes* (group C). The bacterial aerosol was generated using a Devilbiss No. 40 nebulizer. Approximately 2×10^7 organisms from a 24 h culture grown in Todd-Hewitt Broth were administered to the animals over a period of 20 minutes. Immediately following the bacterial aerosol exposure, selected animals were sacrificed in order to determine the total number of bacteria deposited into the lungs at « 0 » time. The remainder of the animals were separated into their appropriate treatment groups and maintained in clean filtered room air for a total of 15 days. Total mortality incidence was determined for the $CdCl_2$-exposed animals and compared to that of controls. The mortality data is presented as percent mortality difference from control.

A second indicator of the effect of Cd on the host's susceptibility to infection was obtained by comparing the average number of days that the experimental and control animals survived after exposure to the Streptococci. This relative mean survival time (RMST) was calculated according to

$$\mathrm{RMST} = \left| \sum_{j}^{15} {}_{j}B_{j} \right| / n$$

where B_j is the number of mice surviving j days and n is the initial number of mice in the experimental group. Here fifteen represents the total length of the experiment.

Bacterial clearance

Forty mice were exposed for 2 h to 325 μg Cd/m³ and then combined with 40 clean air exposed control mice and challenged for 20 min with a bacterial aerosol of streptococci. Eight animals were randomly selected from each treatment group and sacrificed by cervical dislocation at various times post exposure (0 to 6 days). Immediately after sacrifice, a sample of blood from the left ventricle was removed to determine the presence or absence of viable streptococci in the blood of each mouse.

The lungs were then excised, placed in a Sorvall stainless steel homogenizing cup with 1.8 ml of sterile Todd-Hewitt broth, and homogenized for 2 min. Aliquots were removed, diluted, and inoculated on blood agar plates in triplicate. The number of colony forming units was determined following incubation.

Pulmonary cellular population

Rats were exposed to $CdCl_2$ to determine alterations in pulmonary cell populations. The available free cells of the lung were obtained by pulmonary lavage, using the previously described *in vivo* technique [6]. Total white cell counts were determined with a hemocytometer. Viability determinations were performed using the trypan blue dye exclusion method. Differential counts were performed in duplicate by counting 100 cells stained with Giemsa stain.

Statistical methodology

A variety of statistical techniques were used to analyse the effects of Cd on the biological parameters that were measured in these studies. Regression analysis was used to examine the effects of $CdCl_2$ exposure on the susceptibility of mice to increased respiratory infection, relative mean survival time, ratio of dry lung weight to wet lung weight, and Cd/g wet lung and Cd/g dry lung. Correlation between numbers of viable organisms in the lung and their presence in the blood was examined with scatter diagram plots.

Alterations in pulmonary cell population and other parameters, such as body weight and the ratio of dry lung weight to wet lung weight, were analysed by two-way analysis of variance. One factor in this analysis was « $CdCl_2$ exposure concentration » and the other was « post exposure measurement time ». DUNNETT's test was used to elucidate patterns of significant treatment differences [9].

RESULTS

Pulmonary deposition of Cd in mice

The relationship between the $CdCl_2$ aerosol concentrations to which mice were exposed and the resulting pulmonary deposition of Cd was examined for various Cd concentrations ranging from 0 to 2000 $\mu g/m^3$. Regression analysis yielded a statistically significant increasing linear relationship ($p < .0001$ with an R^2 of 87 %) between Cd/g dry lung and Cd aerosol concentration for mice sacrificed immediately after exposure (fig. 1). The averages plotted in figure 1 are also given in table I, along with their standard errors and the number of mice comprising each mean.

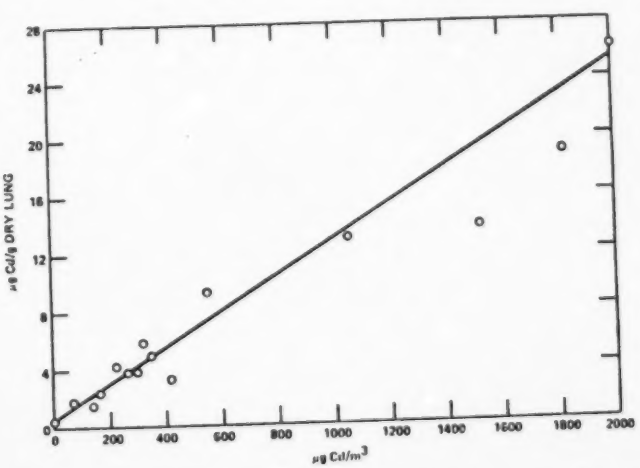

Fig. 1. — Relationship of various concentrations of $CdCl_2$ aerosol to pulmonary deposition of Cd in mice sacrificed immediately after exposure.

Table I also gives summary data concerning effects on the ratio of dry lung weight to wet lung weight and the amount of Cd/g wet lung. When regression analyses were applied to these data, no significant linear relationship was ascertained with changes in $CdCl_2$ aerosol concentration for the ratio of dry lung weight to wet lung weight. This indicates that for the concentration range studied, $CdCl_2$ was not edemagenic. However, results for the amount of Cd/g wet lung were similar to those results obtained by expressing the Cd content on a per gram dry weight basis.

TABLE I

Effects of various concentrations of $CdCl_2$ on mouse lung weights and pulmonary deposition of inhaled metal

Cd ($\mu g/m^3$)	Number of mice	Lung dry wt. / Lung wet wt.	μg Cd / g wet lung	μg Cd / g dry lung
0	65	.219 ± .002	.12 ± .03	.53 ± .12
74	61	.222 ± .001	.38 ± .04	1.71 ± .18
140	7	.224 ± .004	.36 ± .08	1.60 ± .35
165	20	.226 ± .002	.54 ± .09	2.36 ± .37
225	13	.219 ± .003	.96 ± .12	4.36 ± .55
265	21	.225 ± .003	.87 ± .09	3.85 ± .39
300	23	.221 ± .002	.88 ± .12	3.96 ± .51
320	8	.222 ± .002	1.33 ± .07	5.97 ± .32
350	15	.223 ± .003	1.08 ± .10	4.88 ± .47
420	8	.217 ± .003	.70 ± .07	3.22 ± .33
550	7	.204 ± .003	1.89 ± .17	9.30 ± .89
1050	3	.226 ± .006	2.90 ± .76	12.88 ± 3.35
1520	8	.226 ± .001	3.10 ± .50	13.66 ± 2.15
1830	8	.218 ± .002	4.10 ± .28	18.84 ± 1.30
1990	48	.224 ± .002	5.95 ± .22	26.64 ± .97

Data for all response variables are mean values ± standard error as determined immediately after exposure.

Infectivity experiments

The mortality difference between $CdCl_2$-exposed and control mice demonstrated a statistically significantly increasing linear relationship with the logarithm of the inhaled $CdCl_2$ aerosol concentration (fig. 2). Correspondingly, this increasing toxicity of $CdCl_2$ with increasing concentration is reflected in a significant negative linear relationship for relative mean survival time. Figure 2 also shows that the 95 % confidence interval for these two response parameters excludes zero for $CdCl_2$ concentrations which exceed approximately 100 $\mu g/m^3$. Thus, 100 μg $CdCl_2/m^3$ is the lowest concentration for which there is evidence of a significant difference from control with respect to enhancement of mortality and reduction of average length of survival for mice challenged with viable streptococci immediately after exposure to $CdCl_2$.

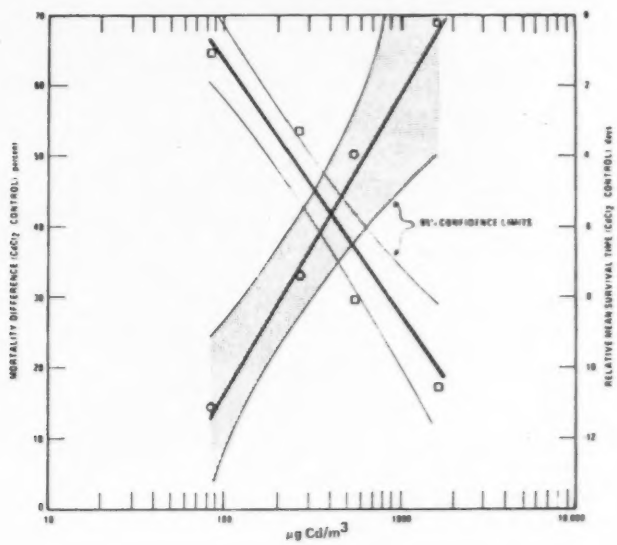

Fig. 2. — Mean difference in mortality and survival time between control and $CdCl_2$ exposed mice.

In a few experiments, the animals were allowed to recover in clean air for 24 h before the bacterial challenge was administered. The results of these experiments are given in table II. Although there were not enough experiments to analyse statistically, it appears that, even with 24 h of recovery before challenge, increased mortality and decreased survival times may be associated with exposure to the two highest $CdCl_2$ concentrations used.

TABLE II

Response to streptococci challenge when administered 24 hours post exposure

Cd (µg/m³)	No. of experiments	Difference ($CdCl_2$ — control) Mortality (%)	RMST (days)
85	1	21	— .3
265	4	2 ± 9.3	— .3 ± .75
550	1	31	— 3.6
1675	2	61 ± 10.8	— 7.1 ± .56

Data are mean values ± standard error. RMST = relative mean survival time.

Clearance of streptococci from the lung

The ability of mice to clear streptococci after a 2 h exposure to 325 μg Cd/m³ is depicted in figure 3. The lungs of both control and $CdCl_2$ exposed mice had an average initial deposition of 700 organisms/lung. Control mice continued to clear streptococci from the lung over a four-day period. However, in the $CdCl_2$ exposed mice, the number of viable streptococci decreased up to 4 h after exposure, and then began to increase markedly. The average number of viable streptococci/lung was 10^5 four days after the cessation of $CdCl_2$ exposure.

Complete summary statistics are given in table III for the lung clearance data just described. It was not possible to accurately determine the lung clearance

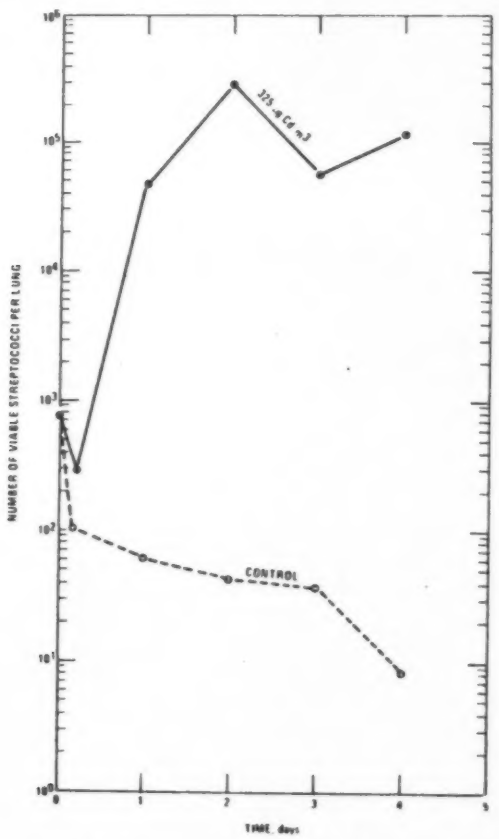

Fig. 3. — Mean number of viable streptococci isolated from lungs of control and $CdCl_2$ exposed mice.

TABLE III

Summary data for quantitation of number of viable streptococci in the lungs of control and $CdCl_2$ exposed mice

Days post exposure	Control		325 μg Cd/m³	
	Sample size	$\log_{10}$ titre	Sample size	$\log_{10}$ titre
0.0	13	2.86 ± .04	15	2.83 ± .05
0.2	6	2.05 ± .24	4	2.44 ± .17
1.0	6	1.78 ± .60	6	4.65 ± .50
2.0	6	1.59 ± 1.00	6	5.44 ± 1.19
3.0	14	1.56 ± .62	14	4.71 ± .94
4.0	14	.97 ± .72	12	5.07 ± 1.26

Data are mean values ± standard error. Logarithmic transformation was used after adding one to all titre values in order to account for mice which had completely cleared the streptococci.

pattern over a longer time period since too few animals survived exposure to 325 μg Cd/m³ for statistical comparison. This concentration produced approximately a 40 % mortality increase over control with an average survival time of 6 days less than control (fig. 2).

Time course of the infection

In order to establish the time course of the laboratory induced respiratory infection with respect to the pathogenesis of the disease, an additional experiment was conduced using this same $CdCl_2$ concentration (325 μg Cd/m³). A scatter diagram of this data is given in figure 4. Note that there is a good correlation between the number of organisms in the lung and the invasion of streptococci into the blood, since negative blood cultures occur with all lung bacterial counts less than 10^4 and positive blood cultures occur with all lung bacterial counts greater than 10^5. This data indicates that when the total number of micro-organisms in the lung reaches a critical level (approximately 10^5), bacterial invasion of the blood occurs. The gray zone in figure 4 represents some animals that may either recover or develop bacteremia and die.

Pulmonary deposition of Cd in rats

Table IV describes the data for rats on the same parameters as for mice (table I) at concentrations of 0, 500, or 1500 μg Cd/m³. Analysis of variance failed to detect any significant alterations in body weight among the three treatment groups for both periods of observation.

TABLE IV

Effects of various concentrations of $CdCl_2$ on rat body weights, lung weights, and pulmonary deposition of inhaled metal

Hours post exposure	Cd ($\mu g/m^3$)	No. of rats	Body wt. (g)	Lung dry wt. / Lung wet wt.	µg Cd / g wet lung	µg Cd / g dry lung
0	0	14	301.0 ± 4.6	.225 ± .002	.08 ± .02	.37 ± .10
	500	8	294.6 ± 8.5	.229 ± .002	1.47 ± .10*	6.45 ± .44*
	1500	20	308.5 ± 6.4	.232 ± .002*	2.50 ± .21*	10.87 ± .94*
24	0	13	302.3 ± 6.1	.221 ± .002	.06 ± .03	.28 ± .11
	500	8	311.2 ± 8.2	.218 ± .002	1.60 ± .09*	7.36 ± .43*
	1500	19	305.3 ± 6.9	.215 ± .001*	1.27 ± .17*	5.96 ± .80*

Data for all response variables are mean values ± standard error. * This mean is significantly different ($p < .05$) from control using DUNNETT's test [9].

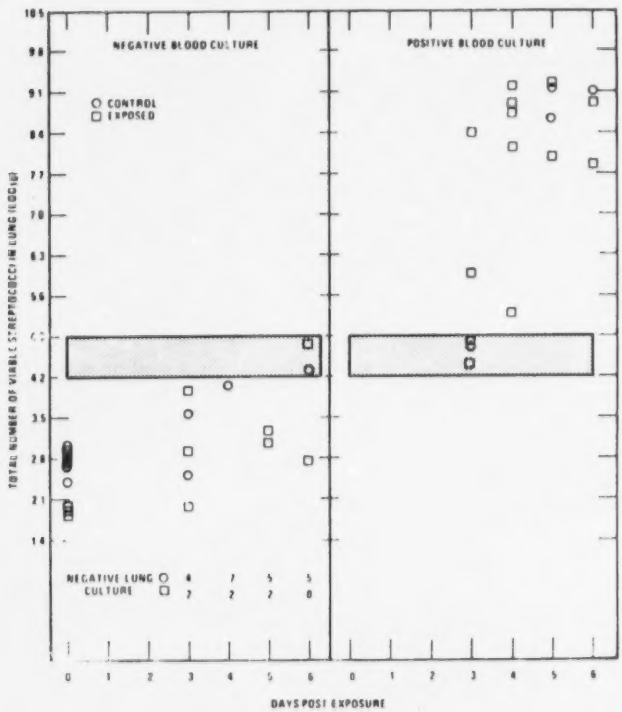

Fig. 4. — Correlation between number of viable lung organisms and the presence of streptococci in blood of mice exposed to 325 µg Cd/m³.

The dry lung weight to wet lung weight ratio for rats sacrificed 24 hours after exposure to 1500 µg Cd/m³ is significantly lower than that observed in control animals. This difference reflects a 2.7 % increase in wet weight. The biological implications of such a small increase have not been determined. There was a significant increase in Cd content in the lung on both a wet and dry weight basis among the three treatment groups for rats sacrificed immediately after cessation of the exposure. A reduction in Cd content 24 hours post exposure to 1500 µg Cd/m³ reflects pulmonary clearance. Clearance was not evident over this same time period for the 500 µg Cd/m³ exposure group.

Pulmonary cellular response experiments

Since there was a significant relationship between aerosol concentration and pulmonary deposition of Cd, other studies were conducted to examine pulmonary cellular responses to this pollutant. Table V presents a complete summary of

TABLE V

Variation in pulmonary cells recovered by lavage after exposure to $CdCl_2$

Hours post exposure	Cd ($\mu g\ m^{-3}$)	No. of rats	Lymphocytes (millions)	Polymorpho-nuclear leucocytes (millions)	Alveolar macrophages (millions)	Total cells (millions)	Viability (percent)
0	0	25	.07 ± .02	.12 ± .03	5.56 ± .32	5.57 ± .33	91.0 ± .98
	500	14	.10 ± .02	.08 ± .03	5.44 ± .46	5.61 ± .46	93.1 ± 1.24
	1500	23	.15 ± .05	1.65 ± .78	3.70 ± .26	4.82 ± .90	79.8 ± 2.96
24	0	23	.08 ± .02	.11 ± .03	5.35 ± .48	5.54 ± .50	92.0 ± 1.16
	500	14	.16 ± .02	.45 ± .13	5.99 ± .39	6.59 ± .48	94.5 ± .95
	1500	26	.83 ± .18	13.06 ± 1.43	5.33 ± .54	19.26 ± 1.68	87.0 ± 1.60

Data are mean values ± standard error.

the raw data for these parameters. Since analysis of variance detected a significant interaction between $CdCl_2$ concentration and post exposure period for all parameters except viability, comparisons among $CdCl_2$ treatment groups were determined separately for each observation period.

Analysis of variance detected a statistically significant decrease ($p < .05$) of 11.2 % in viability of AM of rats exposed to 1500 μg Cd/m³, as compared to control, for rats sacrificed immediately after exposure. Although this same comparison for the 24 h post exposure group was still significant, AM viability was recovering to normal levels. At both periods of observation, there was no statistical difference in AM viability between control and 500 μg Cd/m³ exposed animals.

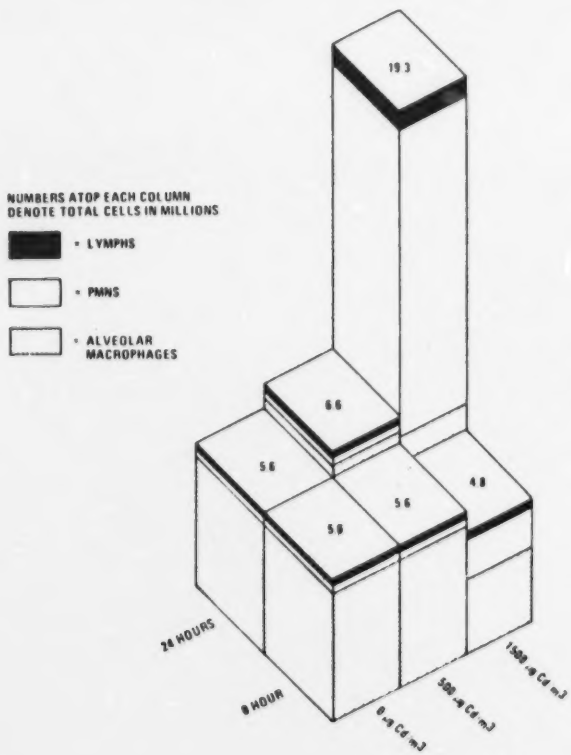

Fig. 5. — Changes in rat pulmonary cell populations in response to $CdCl_2$ immediately and 24 hours after a 2-hour exposure.

The cell composition data given in table V is graphically depicted in figure 5. The statistically significant ($p < .05$) increase in total cells 24 hours after exposure to 1500 μg Cd/m^3 can be seen to be primarily attributable to an influx of polymorphonuclear leucocytes, although there was also a significant increase in lymphocytes. A significant ($p < .05$) reduction in number of macrophages immediately after cessation of $CdCl_2$ exposure was observed for rats exposed to 1500 μg as compared to control rats and those exposed to 500 μg.

DISCUSSION

It has been shown that the kidney, by renal tubular impairment, is usually the critical organ affected in long-term, low-level Cd exposure and that the respiratory system becomes the critical organ with inhalation of high concentrations of Cd [26]. Inhalation of high concentrations of Cd causes reduced ciliary activity, edema, pneumonitis, emphysema, bronchitis, and pulmonary functional changes [1, 11, 18, 28]. However, little information is available concerning the possible association between respiratory disease and exposure to Cd via ambient air. In ambient air, metal concentrations are generally lower than those used in this study, with the exception of areas in the vicinity of metal-emitting industries.

In Sweden, weekly means of 0.3 μg/m^3 were recorded on several occasions 500 m from a factory using copper cadmium alloys; at a 100 m distance, the highest concentration found in a 24 h sampling was 5.4 μg/m^3 [12]. In Japan, a 3 day mean of .56 μg/m^3 and a maximum 24 h average of 3 μg/m^3 have been reported at a distance of 500 m from a zinc smelter [24]. In East Helena, Montana, the average concentration during a 3 month sampling period was .29 μg/m^3 at a distance of 800 m from a smelting plant; the maximum 24 h value was .7 μg/m^3 [19].

Exposure via inhalation does not contribute significantly to the total dose, as compared to the amount absorbed from food or water [29]. However, it is possible that route of exposure has a significant impact on general toxicity, as well as manifestations of toxicity at a specific site. Therefore, when considering the health effects of air pollutants such as Cd, it is essential that the route of natural exposure be explored experimentally before toxicological conclusions are made. Since an individual exists in a multi-stress environment, it is also important to test the consequences of another burden — such as bacteria.

As shown in this manuscript, if a test group subsequently encounters an exposure to a viable infectious agent, the risk of the original pollutant assault becomes much greater. The host must now defend itself from an infectious agent which is benefited by a multitude of subtle effects attributable to the pollutant exposures, *i.e.* altered AM, hyperplasia of epithelial lining cells, edema, and reduced mucous-ciliary clearance. Thus, it is necessary to be cognizant that in toxicological studies there are many factors which can interact and influence the toxicity of the metal. The data obtained in this present study supports the above hypothesis. Using streptococci as an infectious agent, exposure to Cd

concentrations ranging from 100 to 1600 μg/m³ for 2 h caused enhancement of respiratory infection.

The increase in respiratory infections described herein could have been predicted from earlier studies. The authors have previously reported that, under *in vitro* exposure conditions, $CdCl_2$ not only significantly reduced the number of viable AM in culture but also impaired their phagocytic activity [14, 30]. KOSHI and SAKABE [22] used intraperitoneal macrophages and cultured fibroblasts to study the *in vitro* toxicity of metal particles. These authors also reported that exposure to Cd produced high cellular toxicity, as determined by the incorporation of L - (^{14}C) leucine or (^{3}H) thymidine in these isolated cells. The AM, by virtue of its phagocytic activity, constitutes an essential defensive barrier against respiratory infections. Any alteration in the number, viability, or functional capability of these cells renders the host more susceptible to infectious disease.

If the host defense mechanisms are functioning normally, there is a rapid inactivation of the deposited micro-organisms *in situ* [17, 20, 27]. However, if the test substance is capable of inhibiting the lung's ability to destroy the bacteria, an acceleration in the rate of bacterial growth has been demonstrated [5, 13, 16]. In this study Cd produced a similar response.

The clearance of viable organisms from the lung was greatly hindered in the Cd test group. When the number of viable micro-organisms reached a critical level within the lung — approximately 10^5 — the organisms could be identified in the circulatory system. The percentage of mice dying on a given day in these infectivity studies closely followed the daily estimate of the percent of positive blood cultures.

Other investigations have shown that Cd can enhance a host response to infectious agents. EXON *et al.* [10] exposed mice to $CdCl_2$ in drinking water. The concentrations employed did not produce clinical signs of Cd poisoning but a significant increase in mortality to *Hexamita muris* was observed. COOK *et al.* [7] demonstrated a 1000-fold increase in the susceptibility of rats to bacterial challenge after an acute dose of cadmium acetate. In these studies the Cd, as well as the infectious agent (*E. coli*), were administered intravenously.

Cd also has been shown to alter the immune defense status of test animals. KOLLER [21] demonstrated a marked depression of immune response to pseudorabies virus in rabbits maintained chronically on drinking water containing Cd. In the experiments referenced above, EXON *et al.* [10] also showed decreased numbers of antibody producing lymphocytes isolated from spleens of Cd exposed mice. GRAHAM *et al.* [15] studied the effect of a 2 h exposure of $CdCl_2$ (using aerosol techniques identical to those described in this paper) on the number of antibody-producing cells in the spleens of mice. Significant immunosuppression was observed following exposure to 200 μg Cd/m³. In view of these results, it would seem to be important to determine if Cd could also alter pulmonary immune systems, thereby further depressing defenses against infectious respiratory disease.

Thus, in view of the increasing awareness of the effects of metals on host susceptibility to bacterial and viral infections, epidemiological studies designed

to measure incidence rates of respiratory infectious disease appear to be warranted. Much higher Cd air concentrations have been found in the vicinity of known emission sources worldwide, as compared to general urban levels.

Acknowledgment :

The authors wish to ackrowledge the excellent technical assistance of Gloria DEAN, who performed the atomic absorption analysis for these studies.

Disclaimer statement :

This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmenial Protection Agency, and approved for publication. Mention of trade names or commercial projects does not constitute endorsement or recommendation for use.

REFERENCES

1. ADALIS (D.), GARDNER (D.), MILLER (F.), COFFIN (D.). — Toxic effects of cadmium on ciliary activity using a tracheal ring model system. *Envir. Res.*, 1976, in press.
2. AMDUR (M.O.). — The effect of aerosols on the response to irritant gases. *In :* Inhaled Particles and Vapours, Vol. 1, C.N. Davies, ed., Pergamon Press, London, 1961, pp. 281-292.
3. BOREN (H.). — Carbon as a carrier mechanism for irritant gases. *Arch. environm. Hlth*, 1964, **8**, 119-124.
4. BUCHARD (J.). — The significance of particulate emissions. *J. Air Poll. Control Ass.*, 1974, **24**, 1141-1142.
5. COFFIN (D.), GARDNER (D.). — Interaction of biological agents and chemical air pollutants. *Ann. occup. Hyg.*, 1972, **15**, 219-234.
6. COFFIN (D.), GARDNER (D.), HOLZMAN (R.), WOLOCK (F.). — Influence of ozone on pulmonary cells. *Arch. environm. Hlth*, 1968, **16**, 633-636.
7. COOK (J.), HOFFMANN (E.), DILUZIO (N.). — Influence of lead and cadmium on susceptibility of rats to bacterial challenge. *Soc. exp. Biol. Med.*, 1975, **150**, 741-747.
8. DAVIDSON (R.), NATUSCH (D.), WALLACE (J.), EVANS (C.) Jr. — Trace elements in fly ash, dependence of concentration on particle size. *Envir. Sci. Technol.*, 1974, **8**, 1107-1113.
9. DUNNETT (C.). — A multiple comparison procedure for comparing several treatments with a control. *Amer. Stat. Ass. J.*, 1955, **50**, 1096-1121.
10. EXON (J.), PATTON (N.), KELLER (L.). — Hexamitiasis in cadmium-exposed mice. *Arch. environm. Hlth*, 1975, **30**, 463-464.
11. FRIBERG (L.), PISCATOR (M.), NORDBERG (G.). — Cadmium in the Environment. Chemical Rubber Co. Press, Cleveland, 1971, chapter 5, pp. 69-77.
12. FRIBERG (D.), PISCATOR (M.), NORDBERG (G.), KJELLSTROM (T.) eds. — Cadmium in the Environment. Chemical Rubber Co. Press, Cleveland, Ohio, 1974, chapter 3, pp. 9-21.

13. GOLDSTEIN (E.), TYLER (W.), HOEPRICH (P.), EAGLE (C.). — Ozone and the antibacterial defense mechanisms of the murine lung.
Arch. intern. Med., 1971, **127**, 1099-1102.

14. GRAHAM (J.), GARDNER (D.), WALTERS (M.), COFFIN (D.). — Effect of trace metals on phagocytosis by alveolar macrophages.
Infect. Immun., 1975, **11**, 1278-1283.

15. GRAHAM (J.), MILLER (J.), DANIELS (M.), PAYNE (E.), GARDNER (D.). — Influence of environmental trace metals on primary antibody production.
Envir. Res., 1976, submitted for publication.

16. GREEN (G.). — The response of the alveolar macrophage system to host and environmental changes.
Arch. environm. Hlth, 1969, **18**, 548-550.

17. GREEN (G.), KASS (E.). — The role of the alveolar macrophage in clearance of bacteria from the lung.
J. exp. Med., 1964, **43**, 769-776.

18. Health hazards in the manufacture of alkaline accumulators with special reference to chronic cadmium poisoning.
Acta med. scand., 1950, **138** (suppl.), 240.

19. HUEY (N.). — Survey of airborne pollutants. *In :* Helena Valley, Montana, Area Environmental Pollution Study, U. S. Environmental Protection Agency, Research Triangle Park, N.C., 1972, Pub-AP-91, pp. 25-60.

20. KIM (M.), GOLDSTEIN (E.), LEWIS (J.), LIPPERT (W.), WARCHAUER (D.). — Murine pulmonary alveolar macrophages : rates of bacterial ingestion, inactivation, and destruction.
J. infect. Dis., 1976, **133**, 310-320.

21. KOLLER (L.), THIGPEN (J.). — Reduction of antibody to pseudorabies virus in polychlorinated biphenyl-exposed rabbits.
Amer. J. vet. Res., 1973, **34**, 1605-1606.

22. KOSHI (K.), SAKABE (H.). — In : Abstracts of presentation at 45th annual meeting of Japanese Association of Industrial Health, 1974, 110-111.

23. LEE (R.) Jr., von LEHMDEN (D.). — Trace metal pollution in environment.
J. Air Poll. Control Ass., 1973, **23**, 853-857.

24. Ministry of Health and Welfare. — The Opinion of the Ministry and Countermeasures in the Future Concerning Environmental Pollution by Cadmium in the Omuta Area, January 30, 1971, Fukuoka Prefectural Government, Japan, p. 167.

25. NATUSCH (D.), WALLACE (J.), EVANS (C.) Jr. — Toxic trace elements : preferential concentration in respirable particles.
Science, 1973, **183**, 202-204.

26. NORDBERG (G.) ed. — Effects and Dose-Response Relationships of Toxic Metals. Elsevier Scientific Publishing Co., New York, 1976, chapter 5, pp. 38-47.

27. RYLANDER (R.). — Pulmonary defense mechanisms to airborne bacteria.
Acta. physiol. scand., 1968, **306** (suppl.), 1-89.

28. SNIDER (G.), HAYES (J.), KORTHY (A.), LEWIS (G.). — Centrilobular emphysema experimentally induced by cadmium chloride aerosol.
Amer. Rev. resp. Dis., 1973, **108**, 40-48.

29. U. S. Environmental Protection Agency. Scientific and Technical Assessment Report on Cadmium. EPA-600/6-75-003, March, 1975.

30. WALTERS (M.), GARDNER (D.), ARANYI (C.), COFFIN (D.). — Metal toxicity for rabbit alveolar macrophages *in vitro*.
Envir. Res., 1975, 32-47.

RÉSUMÉ

L'exposition au chlorure de cadmium en aérosol tend à réduire nettement la capacité de défense du poumon contre les agressions bactériennes. Une augmentation significative du taux de mortalité a été observée chez des souris exposées à des concentrations de chlorure de cadmium allant de 80 à 1 600 µg/m³, puis soumises à une infection par streptocoques viables. L'accroissement du taux de mortalité va de 15 %, pour la plus faible concentration de $CdCl_2$, à environ 70 %, pour la plus haute concentration.

Afin de déterminer les différents mécanismes susceptibles d'expliquer cette diminution de la résistance à l'infection, divers systèmes de défense pulmonaire ont été étudiés. On constate un abaissement significatif du nombre total des macrophages alvéolaires récoltés à partir des poumons de rat immédiatement après la fin de l'exposition. Le nombre des macrophages revient à la normale (environ 5,5 millions) dans un délai de 24 heures après l'arrêt de l'exposition. Cependant le nombre de leucocytes polynucléaires atteint 1,5 million immédiatement après la fin de l'exposition, et 13 millions dans les 24 heures qui suivent la fin de l'exposition. Le nombre de lymphocytes n'est pas modifié dans ces conditions d'exposition. Les résultats concernant l'épuration pulmonaire des streptocoques après exposition au $CdCl_2$ sont en étroite corrélation avec le taux de mortalité observé.